AWARD NUMBER: W81XWH-14-1-0032

TITLE: Targeting Premalignant Lesions - Implications for Early Breast Cancer Detection and Intervention

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REPORT DATE: April 2017

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
April 2017	Annual	1 Apr 2016 - 31 Mar 2017

4. TITLE AND SUBTITLE

Targeting Premalignant Lesions - Implications for Early Breast Cancer Detection and Intervention

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-14-1-0032

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Breast cancer progression constitutes a multistep process through a series of intermediate hyperplastic and neoplastic stages to invasive carcinoma. In this study, we aimed to identify peptides that specifically recognize premalignant lesions in the mammary tissue. To achieve this goal, we utilized the power of phage display to probe hyperplastic lesions associated with premalignant disease in a transgenic MMTV-PyMT animal model. We have identified a peptide CISQ that targets to the stroma in premalignant lesions and binds to cancer-associated fibroblasts (CAFs) in MMTV-PyMT mice. Considerable numbers of CAFs are frequently observed within the tumor-associated stroma of various human cancers, including those of the breast, prostate, lung, colon and pancreas and have been also reported in the premalignant lesions. This peptide could provide us with an opportunity to therapeutically intervene to successfully inhibit or even reverse tumor progression.

15. SUBJECT TERMS
Early breast cancer, early detection, homing peptides, premalignant lesions, targeted nanomedicine

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	9	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

Difficulty in managing treatment of advanced stage breast cancer has led to the goal for detection and intervention of early-stage disease. However, current non-invasive methods are not specific enough to reliably detect early breast cancer. Our laboratory has successfully employed *in vivo* screening of phage libraries to develop new probes for breast tumors. Progression of breast cancer constitutes a multistep process wherein each stage is characterized by distinct phenotypic changes that occur in the mammary gland. We proposed to utilize this animal model to probe early stage (premalignant) lesions with phage libraries to identify novel peptides that specifically recognize the premalignant stage of breast cancer. These peptides and the identification of their putative receptors will help our understanding of the underlying biology of breast cancer progression. Furthermore, these probes will be used to develop targeted therapeutic nanoparticles for early intervention in breast cancer.

2. KEYWORDS:

Early breast cancer, early detection, homing peptides, premalignant lesions, targeted nanomedicine

3. ACCOMPLISHMENTS:

Major Goals and Objectives approved (and completed) for this project are as follows:

<u>Specific Aim 1:</u> Identify peptides that specifically home to premalignant breast lesions (Months 1-12)

Task 1. To screen phage libraries for new peptides that specifically recognize premalignant lesions (Months 1-9):

- Develop and characterize the CX7C and X7 phage libraries for screening (COMPLETED)
- Screening of libraries in MMTV-PyMT animals (COMPLETED)
- High throughput sequencing on recovered phage from these lesions (COMPLETED)
- Bioinformatics analysis (ONGOING)

Task 2. To validate the homing specificities of individual phage and synthetic peptides (ONGOING)

- Individually test homing of identified phage (COMPLETED)
- Determine phage specificity to premalignant lesions (COMPLETED)
- Phage overlay on human tissue microarrays (To be done)
- Validation of peptide homing in MMTV-NeuYD transgenic mouse model (to be done)

Specific Aim 2: Identify and characterize putative receptors in premalignant lesions (Months 12-24).

Task 1: To identify putative receptors of these peptides in these early lesions (Months 12-15) (COMPLETED)

Task 2: To characterize the identified receptor in early lesions (Months 15-18) (To be done)

Task 3: To study significance of receptor in disease progression across different stages of breast cancer (Months 18-24) (To be done)

Specific Aim 3: Target premalignant lesions utilizing peptide-conjugated nanoparticles to prevent/delay progression of premalignant lesions to invasive breast cancer (Months 18-36)

Task 1: To engineer and characterize peptide conjugated therapeutic nanoparticles (Months 18-24)

- Develop peptide nanoparticle drug conjugates (Months 18-20) (*COMPLETED)*
- Characterize targeted nanoparticles (Months 20-24) (COMPLETED)

Task 2: Study the effect of targeted delivery of therapeutic nanoparticles on the onset of the disease (Months 24-36)

- Treat MMTV-PyMT animals with peptide nanoparticle conjugates (*Months 24-32*) (To be done)
- Evaluate tumor progression (*Months 32-36*) (To be done)

RESULTS:

Specific Aim 2: Identify and characterize putative receptors in premalignant lesions

As part of this Aim 1 of this grant, we previously identified peptides that home to premalignant lesions in the MMTV-PyMT model. Two of these peptides on intravenous administration showed localization to the ECM in the premalignant tissues. Fig. 1 shows the two peptides, CSG and CAGK peptide localizing in matrix of PyMT mice but not in age-matched wild type mice. This data suggested that the ECM undergoes change starting from the premalignant stage. There has been a previous report that showed that matrix crosslinking forces breast tumor progression (*Cell* 139.5 (2009): 891–906).

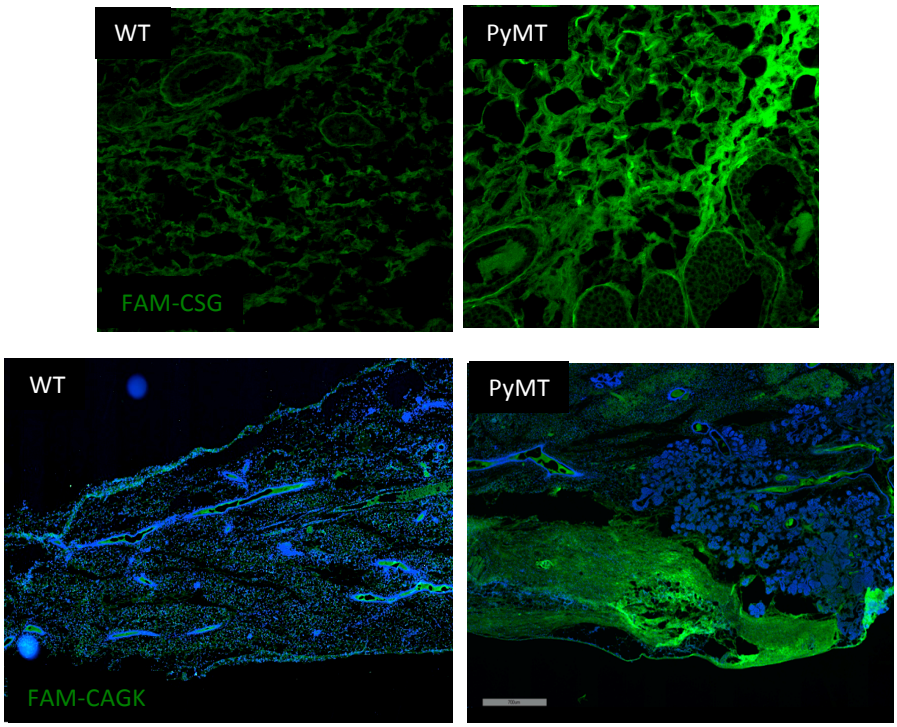

Fig. 1. Peptide homing to premalignant lesions. Immunofluorescence staining on mammary sections from mice injected with CSG peptide (top panel) and CAGK peptide (bottom panel). Both peptides show localization to premalignant lesions and not to normal breast tissue.

As a next step, we decided to study the global expression of ECM proteins in the premalignant stage. For this we isolated ECM proteins from PyMT and WT mice at day 75 using a protocol described by Naba et al. in *J. Vis. Exp. (101), e53057, doi:10.3791/53057 (2015).* The results from the proteomics analysis on these samples are summarized in Table 1. In agreement with our peptide homing data, many ECM proteins such as Elastin, Collagen, Nidogen showed a dramatic increase in PyMT over the WT mice (last column in Table 1). This was further confirmed by immunofluorescence staining of Collagen IV in PyMT and WT sections (Fig. 2).

Lastly, to demonstrate payload delivery to premalignant lesions using these peptides, we conjugated the peptides to silver nanoparticles (AgNP). AgNP platform has been previously used for targeted delivery and is readily available in our lab (*Braun et. Nature Materials, 2014)*. Both iRGD-AgNP and CAGK-AgNP targeted the premalignant lesions in PyMT mice (whereas control peptide conjugated AgNP did not show any specific accumulation (Fig. 3). No AgNP homing was observed in the WT mice. This data demonstrate that these peptides can mediate delivery of nanoparticle payloads to the premalignant lesions in the PyMT mice.

Protein IDs	Protein names	Gene names	Intensity	Log2FC
P54320	Elastin	Eln	90192000	25.38703166
Q60847	Collagen alpha-1(XII) chain	Col12a1	53855000	24.08848541
O88322	Nidogen-2	Nid2	3378500	20.68168833
Q02788	Collagen alpha-2(VI) chain	Col6a2	887470000	5.41470701
Q60675	Laminin subunit alpha-2	Lama2	80975000	4.737946445
P02468	Laminin subunit gamma-1	Lamc1	380820000	4.432539311
Q63870	Collagen alpha-1(VII) chain	Col7a1	615510000	3.488094343
Q61292	Laminin subunit beta-2	Lamb2	218130000	3.485497297
Q61001	Laminin subunit alpha-5	Lama5	108510000	3.293508859
P02463	Collagen alpha-1(IV) chain;Arresten	Col4a1	120540000	3.031439123
P11276	Fibronectin;Anastellin	Fn1	88187000	2.500048499
Q61789	Laminin subunit alpha-3	Lama3	19271000	2.410435191
Q62009	Periostin	Postn	2.17E+09	2.308374049
Q3U962	Collagen alpha-2(V) chain	Col5a2	810140000	2.223170359
Q61555	Fibrillin-2	Fbn2	62846000	2.15626615
P10493	Nidogen-1	Nid1	34302000	1.672243114

Table. 1. Proteomics analysis of ECM protein in premalignant lesions. ECM proteins listed in decreasing order of fold change denoted by Log2FC column. Log2FC was calculated as fold change between PyMT and WT samples on a log2 scale.

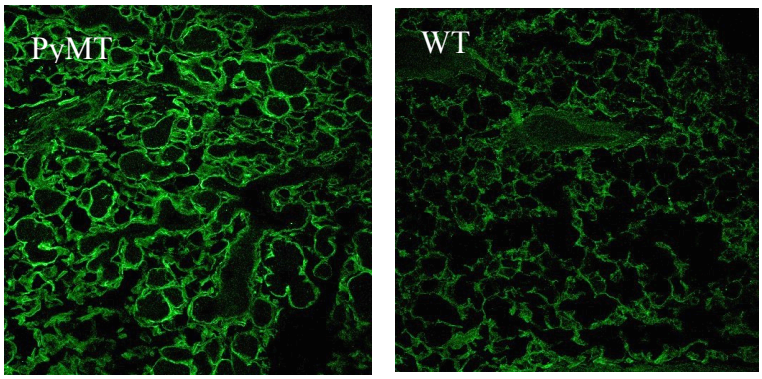

Fig. 2. Collagen IV expression in PyMT and WT glands. Immunofluorescence staining for Collagen IV shows higher expression in PyMT mammary sections than WT mice.

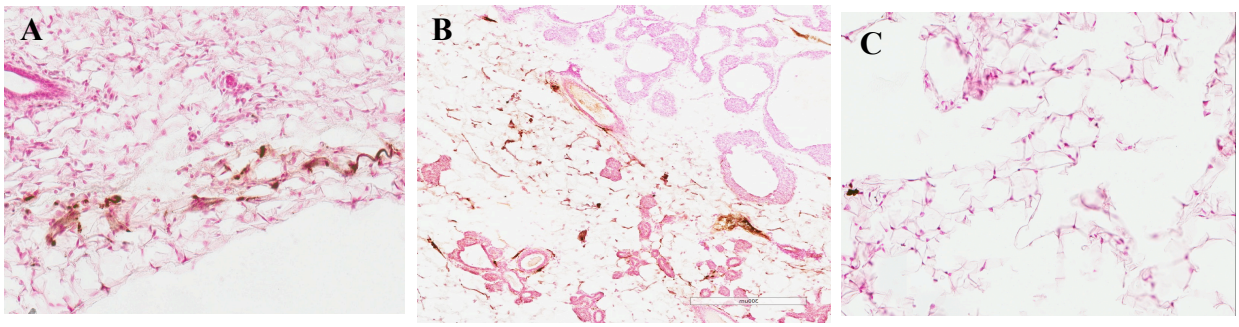

Fig. 3. Peptide mediated nanoparticle delivery to premalignant lesions. Peptide conjugated AgNP were injected intravenously in mice and mammary glands were isolated and analyzed for nanoparticle accumulation by silver staining (brown). A. iRGD-AgNP in PyMT lesions. B. CAGK-AgNP in PyMT lesions. C. Control AgNP in PyMT lesions.

Opportunities for training and professional development:
Appointed as a founding member of the Sanford Burnham Prebys Postdoc Training Advisory Group to strategize in developing training strategies for postdocs at SBP.

Dissemination of results:

- Participated in collaborative meetings with Hamzah group in Australia and Tessalu group in Estonia (see collaborations).
- Registered to attend the 16th Annual International Congress on the Future of Breast Cancer® West in July 2017.

4. IMPACT:

Impact on the development of the principal discipline(s) of the project: Demonstrated targeted delivery of nanoparticle payload to premalignant lesions of breast cancer. This approach can be applied to delivery of therapeutics to these lesions as a method of early intervention of breast cancer.

Impact on other disciplines: The homing property of the peptide to premalignant lesions can be utilized for diagnostic applications. This can be done by combining the peptide with imaging agents such as iron oxide nanoparticles for non-invasive detection of these premalignant lesions.

Impact on technology transfer: Nothing to report

Impact on society beyond science and technology: Nothing to report

5. CHANGES/PROBLEMS - Nothing to report

6. PRODUCTS:

Journal publications. 1 manuscript on breast cancer submitted for publication, 2 manuscripts in preparation

Books or other non-periodical, one-time publications: *None*

Website(s) or other Internet site(s): None

Technologies or techniques – Peptides that can target premalignant lesions

Inventions, patent applications, and/or licenses - None

Other Products - None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name: Aman Mann
Project Role: PI
Nearest Person Month Worked: 12
Contribution to Project: Principal Investigator and oversee all scientific, experimental and administrative aspects

Name: Erkki Ruoslahti
Project Role: Mentor
Nearest Person Month Worked: 0
Contribution to Project: Serves as a mentor to Dr. Aman Mann

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: None